amended to more specifically delineate the claimed invention. Claim 13 has been amended to more distinctly claim the subject invention. Claim 17 has been amended to remove the term "modified." Claim 20 has been added. Support for new claim 20 can be found on page 15, lines 1-2. Applicant notes the Examiner's request to comply with the sequence rules under 37 CFR 1.821-1.825 and will file a completed sequence listing within the statutory period.

Summary of the invention

The subject invention relates to a novel nucleic acid construct for delivery of antisense sequences for suppressing target gene expression. The construct includes a pol II promoter region, a 5' and a 3' unmodified U snRNA stem loop structure, and an antisense region located between the 5' and the 3' stem loop structures. Optionally, a ribozyme and/or a 5' trimethylguanosine cap are included in the construct.

Restriction Requirement

Applicants affirm the provisional election of the claims of Group I (claims 1-13, 15, and 17) drawn to a nucleic acid construct) with traverse and cancel claims 14, 18, and 19 without prejudice to renewal. Claim 14 has been placed in Group II, drawn to a nucleic acid construct and method of introducing said construct in vivo. Group II also includes Claims 1-13 and 17, which can also be classified in Group I (the elected invention). Claim 16 has been placed in Group III, which also includes claims 1-13 and 17). Applicants respectfully submit that if Claim 14 can be grouped with Claims 1-13 and 17, and Claim 16 can be grouped with Claims 1-13 and 17, the search required for the subject matter of Group I (Claims 1-13, 15, and 17) must include the subject matter of Group II(Claims 1-14, and 17) and Group III (Claims 1-

13, 16, and 17). Thus, examining the claims of groups I , II, and III would pose no undue burden upon the Examiner.

In view of these remarks, the Examiner is thus respectfully requested to withdraw the restriction requirement and examine Claims 1-17 in the present application.

Rejections Under 35 U.S.C. §112, first paragraph

Claims 1-13, 15, and 17 are rejected for allegedly not delineating the relationship between the 5' and 3' stem loops and the antisense nucleic acid. Applicant respectfully traverses this rejection.

The Office Action states that the specification is enabling for "a nucleic acid construct comprising 5' and 3 U snRNA stem loop structures and a nucleic acid which is antisense to a gene of interest, wherein the antisense nucleic acid is between said 5' and 3' stem loops." Claims 2 and 4 have been canceled, and the amendments to claims 1,3, 5-7, 12, 13, and 17 to more distinctly point out the relationship of the 5' and 3' stem loops and the antisense nucleic acid, are believed to render this rejection moot.

Claims 1-13, 15, and 17 stand rejected for the breadth of the term "antisense nucleic acid," which the Office Action states could be directed to something not within the gene of interest. Applicant respectfully traverses this rejection.

Claims 2 and 4 have been canceled. Claims 1 has now been amended to recite "an antisense nucleic acid sequence directed against a target of interest." The Office action states that "the breadth of the claim is non-enabling with respect to the anti-sense nucleic acid." The pessimistic statements about antisense nucleic acids presented in the Office Action are not an indication that antisense will not work. The potential of antisense oligonucleotides to

selectively inhibit protein synthesis from a target gene of interest has generated a great deal of enthusiasm for their development of experimental therapeutics (e.g. McShan, W.M., et al. (1992) J. Biol. Chem. 267:5712-21; Calbretta, B. (1991) Cancer Research 51:4505-4510), and the production of antisense oligonucleotides has further been a subject of patents (e.g. Noonberg et al., U.S. Patent 5,624,803.)

The specification provides ample guidance for production of antisense oligonucleotides and triple helix oligonucleotides. Furthermore the specification recites methods for introducing the antisense molecules into cells (page 13, lines 3 to page 14, line 7). Applicants submit that the introduction of nucleic acids into cells (across the membrane) is routine to one skilled in the art, and that the methods of calcium phosphate, liposome /DNA complexes, and ligand/DNA conjugates and not limited to specific cell types (for review see Cooper, M.J., 1996, "Noninfectious Gene Transfer and Expression Systems for Cancer Gene Therapy," *Seminars in Oncology* 23(1):172-87).

In addition, a number of methods are known in the art for determining whether a particular antisense reagent will inhibit gene expression. Screening of candidate oligonucleotides by any one of these methods requires no more than routine screening, and is not undue experimentation. The following articles demonstrate the level of skill in the art for selection of appropriate antisense reagents. Duroux *et al.* (1995) N.A.R. 23:3411-3418, entitled "*Rational design of point mutation-selective antisense DNA targeted to codon 12 of Ha-ras mRNA in human cells*". Hyndman *et al.* (1996) Biotechniques 20:1090-1094, entitled "*Software to determine optimal oligonucleotide sequences based on hybridization simulation data*". DeDionisio and Lloyd (1996) J. Chromatogr. A. 735:191-208, entitled "*Capillary gel electrophoresis and antisense therapeutics. Analysis of DNA analogs*". Ho *et al.* (1996) N.A.R.

24:1901-1907, entitled "*Potent antisense oligonucleotides to the human multidrug resistance-1 mRNA are rationally selected by mapping RNA-accessible sites with oligonucleotide libraries*".

Using the art-established methods for selection of oligonucleotides and the direction provided by the subject application, a person of ordinary skill in the art could practice the claimed invention without undue experimentation. Withdrawal of the rejection is requested.

The Examiner rejects claims 8-11, citing a references indicating various difficulties in the practice of ribozyme techniques. However, difficulties alone do not render an application non-enabling: enablement does not require a guarantee of success or an absence of obstacles. All therapies encounter problems in development. Applicants note that a number of antisense/ribozyme compositions are currently in clinical trials, including treatments for CMV, genital warts, kidney transplant rejection, autoimmune diseases such as rheumatoid arthritis, chronic myelogenous leukemia, and AIDS. An antisense treatment for AIDS is currently in Phase IB/II trials (Hybridon GEM® 91) and an antisense treatment for CMV is currently in Phase I/II trials (Hybridon GEM® 132). Applicants respectfully request withdrawal of the rejection.

Claim 12 is rejected for reciting specific antisense nucleic acids. The Office Action states that no direction or guidance is presented to enable one of skill in the art to make the construct for some of the genes. Applicants respectfully traverse. A construct of the invention which targets RENT1 is described in the specification on page 14, lines 16-19, and is shown in Figure 1). A construct which targets fibrillin expression is described in the specification on pages 23, line 7 to page 25, line 20, and the effectiveness of this construct is further described on page 25 line 21 to page 27, line 25. Examples of other useful constructs are described in the specification on page 9, line 7 to page 10, line 2. Given the number of methods

well-known in the art for screening oligonucleotides (see above), applicants submit that ample guidance is available to make any construct, especially RENT-1, HPV E6, HIV, hyaluronic acid synthase, and fibrillin, which are expressly disclosed in the specification. Withdrawal of the rejection is requested.

Rejection Under 35 U.S.C. 112, second paragraph

Claim 1 is rejected for not reciting a specific range of suppression. While Applicant respectfully traverses this rejection, Claim 1 has now been amended to recite "wherein said expression of said target gene is suppressed by at least 75% of the normal level of expression" rending this rejection moot.

Claims 2 and 3 are rejected for the use of the term "unmodified." Claim 2 has been canceled. However, the limitations of claim 2 have been incorporated into claim 1, thus this rejection will be addressed as it applies to amended claim 1 and to dependent claim 3.

Applicants respectfully traverse, and submit that the term "unmodified" is adequately defined in the specification on page 7, lines 17-23, as follows:

> "The term "unmodified" means that the folding pattern of the stem loop structure is not compromised by alterations in the nucleic acid sequence of the naturally occurring molecule."

Examples of what would constitute an "unmodified" stem loop are also given on page 7. Applicants would like to remind the examiner of MPEP section 608.01(o), which states:

> "A term used in the claims may be given a special meaning in the description. No term may be given a meaning repugnant to the usual meaning of the term."

Applicants submit that the term "unmodified" is described in the specification in accordance with

MPEP 608.01(o),and thus removal of the rejection is respectfully requested.

Claim 12 has been rejected for being indefinite in it's recitation of "..wherein the antisense nucleic acid is selected from the group consisting of..." Claim 12 has been amended to more distinctly point out the Applicant's invention, rendering this rejection moot.

Claim 13 is rejected for it's use of the term "suppressive-effective amount." Applicants submit that the term is defined on page 15, lines 4-13, and that the use of the term "suppressive-effective" is in accordance with MPEP 608.01(o) (see above.) Thus Applicants respectfully request withdrawal of the rejection.

Claim 13 is rejected for the limitation "the gene" in line 3. This rejection is rendered moot by the amendments to claim 13.

Claim 17 is rejected for being indefinite for the use of the term "modified." Claim 17 has been amended to more distinctly claim the Applicant's invention, rendering this rejection moot.

Rejections Under 35 U.S.C. §102(e)

Claims 1, 2, 4, 6, 8, 9, 13, and 15 are rejected as allegedly being anticipated by Noonberg et al. (U.S. Patent 5,624,803). Claims 2 and 4 have been canceled. This rejection is traversed as applied to claims 1, 6, 8, 9, and 13. Applicant respectfully traverses this rejection.

Noonberg et al. describes methods and constructs for delivering antisense, triplex, and or/ribozyme oligonucleotides intracellularly using U6-type RNA pol III based constructs which are termed "oligonucleotide generators." The constructs of Noonberg et al. includes a pol III promoter, a stabilizing region on the 5' end which can be a hairpin , an antisense sequence, and a termination sequence, where the components can be transcribed by RNA polymerase III to

produce a transcript (see column 8, paragraph 1). A second similar hairpin may be, but is not necessarily at the end of the termination sequence.

The subject invention consists of a pol II based system, not a pol III based system, as disclosed by Noonberg et al. A single prior art reference properly anticipates an invention under 35 U.S.C. §102 only if every element of the claimed invention is identically shown in that reference (*In re Bond*, 910 F.d. 831, 15 USPQ2d 1566 (Fed. Cir. 1990). Since Noonberg et al. Does not teach each and every element of the claimed invention, it cannot anticipate the claimed invention. Accordingly, Applicant respectfully requests withdrawal of the rejection.

Rejections Under 35 U.S.C. §103

Claims 3 and 5 stand rejected under 35 U.S.C. §103 over Noonberg et al. (U.S. Patent 5,624,803). Applicants respectfully traverse the rejection.

Claim 3 limits the stem loop structures described in claim 1 to the U1 snRNA stem loops. Claim 5 limits the pol II promoter described in claim 1 to the U1 snRNA promoter.

As described above, Noonberg et al. disclose methods and constructs for delivering antisense, triplex, and or/ribozyme oligonculeotides intracellularly using U6-type RNA pol III based constructs. The subject invention consists of a pol II based system, not a pol III based system, as disclosed by Noonberg et al. Noonberg et al. teach away from the pol II based system; they describe a pol II based system as transcribing at a low rate, generating varying transcript lengths, and producing transcripts with long polyadenylated tails (seen as undesirable), and not being correctly localized in the cell (see the paragraph that bridges the end of column 17 to the beginning of column 18).

The discussion quoted in the Office Action from column 42 of the Noonberg patent, is directed toward monitoring genes for signs of transcriptional disfunction, not toward the use of the U1 genes in the constructs of Noonberg et al. In this particular example, U1 is monitored in order to document if the U6 based construct specifically inhibits the target. Noonberg et al is describes the selection of U1 because it is nuclear, abundant, constitutively expressed, and stable. Applicant submits that other genes, such as β-actin, could just have easily been used. In the Example, U1 levels are not affected following transfection with the chimeric U6 gene, as expected.

Noonberg et al. also describe many differences between U1 and U6 (e.g., pol II vs. pol III transcription, the presence of a TATA box) in column 42. Although as Noonberg states, U1 is related to U6 as it can form a spliceosome, there are a great many differences between U1 and U6 Examples of these differences include: (1) U1 is more abundant than U6 (2) U1 utilizes RNA pol II, while U6 utilizes RNA pol III, (3) U1 has a 5' and a 3' stem loop structure, U6 has a single stem loop structure, (4) U1 has a trimethylguanosine cap, U6 has a γ-monomethylguanosine cap, (5) U1 is a member of the Sm class (a classification based on recognition by anti-Sm antibodies, all small RNPs known to participate in synthesis, processing, and export to cytoplasm of mature RNAs react with anti-Sm antibodies) while U6 is not, (6) U1 functions independently, U6 requires the presence of U4, (7) U1 is dispersed in the nucleoplasm, while U6 is concentrated in speckles and coiled bodies (Baserga and Steitz, 1993, "The Diverse World of Small Ribonucleoproteins," In: *The RNA World*, Cold Spring Harbor Laboratory Press, New York, pp. 359-381).

Consequently, Applicants submit that the use of U6 does not render the use of U1 obvious, and respectfully request withdrawal of the rejection.

Claims 1-6, 8-10, 12, 13, and 15 stand rejected over Michienzi et al., who describe a U1 snRNA vector for specifically targeting a ribozyme to the nucleus. Applicant respectfully traverses this rejection.

In the constructs of Mchienzi et al. the catalytic core of the ribozyme's hammerhead motif is substituted into the stem-loop III of the U1 snRNA. This location is selected for very specific reasons delineated on page 7220, column 2, first paragraph. Applicants submit that the construct is shown in Figure 1, bottom left, on page 7221. The Office Action states that the construct is depicted in Figure 1, bottom right. Applicants submit that the bottom right portion of the Figure illustrates the pairing of Rev pre-mRNA with U1-Rz-5'. In the bottom right portion, mismatches are shown to occur within a single 5' stem loop structure of U1, and the cleavage sites for the ribozyme within the single, 5' stem loop are indicated (see the Figure legend).

All of the constructs taught by Michienzi et al. have substitutions in the 5' stem loop; no construct has unmodified stem loop structures, which are an essential component of the Applicant's invention. Applicant submits that a construct containing a unmodified, naturally occurring 5' U snRNA stem loop structure, and unmodified, naturally occurring 3' U snRNA stem loop structure, with an antisense sequence located between the two stem loops, would not be obvious from the work of Michienzi et al.

Furthermore, an antisense effect is not desired in the constructs of Michienzi et al.; specific constructs are designed to control for any antisense effect (p. 7220, column 2, paragraph 3). Therefore, Applicants submit that the substitution of the antisense construct for the ribozyme contained in the constructs of Michienzi et al. would not be obvious. Applicants respectfully request that the rejection be withdrawn.

Claims 1, 4, 6, 8, 9, 12, 13, and 15 are rejected over Taira et al. (U.S. Patent 5,500,357). Applicant respectfully traverses this rejection.

Taira et al. describe a recombinant DNA in which a DNA encoding a trans-acting ribozymes of interest is ligated to DNAs encoding another cis-acting ribozymes which serve to cleave the 5' and 3' ends of the trans-acting ribozyme of interest. The system is constructed by connecting the units in tandem. No stem loop structures are contained in the constructs of Taira et al. Applicants would like to point out that the structures shown in Figures 3 and 7b are ribozyme structures, not stem loop structures as suggested in the Office Action. Applicants submit that the tandem ribozyme constructs disclosed by Tara et al. do not render the use of 5' and 3' unmodified, naturally occurring U snRNP stem loops obvious for targeting antisense, and request withdrawal of the rejection.

Claims 1-6, 8-11, 13, and 15 stand rejected over De Young et al., who disclose the targeting of hammerhead ribozymes to atrial natriuretic factor mRNA using the construct $pUHHU_{117}$, which contains the U1 promoter and the U1 3' end, which contains the terminator and the 3' stem loop structure (see page 12129, column 1, figure 3, and page 12136, column 1, last paragraph). Applicant respectfully traverses this rejection. The constructs of De Young et al. do not contain a 5' stem loop structure, nor do they suggest the positioning of an antisense sequence between two U snRNP stem loop structure.

Moreover, De Young et al. specifically state that "..in vitro transcription of the RNA polymerase II-activated U1 promoter is problematic..." (page 12129, column 2, paragraph 2), and they thus used a PCR based approach to produce chimeric DNA which encoded the T7 promoter, the U1 initiation sequence, and the ribozyme and U1 termination sequences. De Young et al. specifically teach away from the use of a pol II promoter, which is an inherent part

of the subject invention. The Applicant submits, therefore, that the work of De Young et al. does not render the subject invention obvious, and respectfully request withdrawal of the rejection.

In view of the comments above and the amendments to the claims, Applicants respectfully request the rejection be withdrawn.

Conclusion

In summary, for the reasons set forth herein, Applicant submits that all of the pending claims are now in condition for allowance, which action is requested.

If the Examiner would like to discuss any of the issues raised in the Office Action, Applicant's representative can be reached at (619) 678-5070.

Please charge any additional fees, or make any credits, to Deposit Account No. 06-1050.

Respectfully submitted,

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